CLAIMS

What is claimed is:

1. A method for determining the predominant physiological effect of a composition comprising hemoglobin, comprising the steps of:
    a) obtaining EPR or UVspectra of iron-nitrosyl hemoglobin derivatives formed by incubation of limiting NO with hemoglobin at various degrees of oxygen saturation;
    b) determining from the results in a) whether the composition shows non-cooperativity or cooperativity in binding of NO to the hemoglobin; and
    c) if the composition shows cooperativity, then assaying the composition at an oxygen saturation at which the hemoglobin is approximately 99% oxyhemoglobin and 1% deoxyhemoglobin, under limiting NO concentration, to determine whether *S*-nitrosohemoglobin or iron nitrosyl-hemoglobin is greater;

    wherein, if the composition shows non-cooperativity, then the predominant physiological effect of the composition is elimination of NO; if the composition shows cooperativity and if *S*-nitroso-hemoglobin is greater, then the predominant physiological effect of the composition is delivering NO; and if the composition shows cooperativity and if iron nitrosyl-hemoglobin is greater, then the predominant physiological effect of the composition is trapping of NO.

2. A method for determining the predominant physiological effect of a composition comprising hemoglobin, comprising the steps of:
    a) obtaining EPR or UVspectra of iron-nitrosyl hemoglobin derivatives formed by incubation of limiting NO with hemoglobin at various degrees of oxygen saturation;

b) determining from the results in a) whether the composition shows non-cooperativity or cooperativity in binding of NO to the hemoglobin; and

c) if the composition shows cooperativity, then assaying the composition at an oxygen saturation at which the hemoglobin is approximately 99% oxyhemoglobin and 1% deoxyhemoglobin, under limiting NO concentration, to determine whether *S*-nitrosohemoglobin or iron nitrosyl-hemoglobin is greater;

wherein, if the composition shows non-cooperativity, then the predominant physiological effect of the composition is vasoconstriction; if the composition shows cooperativity and if the most prevalent species of NO-modified hemoglobin is *S*-nitrosohemoglobin, then the predominant physiological effect of the composition is vasodilation; and if the composition shows cooperativity and if iron nitrosyl-hemoglobin is greater, then the predominant physiological effect of the composition is vasoconstriction.

3. A method for delivering NO to tissues of a mammal, comprising administering to the mammal dinitrosyl iron complex of hemoglobin.

4. A method for producing a composition comprising *S*-nitrosohemoglobin, said method comprising adding NO to a composition comprising oxyhemoglobin.

5. A method for producing a composition comprising intraerythrocytic *S*-nitrosohemoglobin, said method comprising adding NO to a composition comprising oxygenated erythrocytes.

6. A method for producing a composition comprising intraerythrocytic NO at greater than about 50 nM, said method comprising adding NO to a composition comprising oxygenated erythrocytes.

7. A method for producing a composition comprising intaerythrocytic *S*-nitrosohemoglobin, said method comprising adding NO to a composition comprising deoxygenated erythrocytes.

8. A method for producing a composition comprising intraerythrocytic NO at greater than about 50 nM, said method comprising adding NO to a composition comprising deoxygenated erythrocytes.

9. A method for delivering NO in a mammal, comprising administering to the mammal a composition comprising hemoglobin and about 100 millimolar phosphate.

10. A method for treating septic shock in a mammal, comprising administering to the mammal a composition comprising hemoglobin and about 100 millimolar phosphate.

11. A method for trapping NO as iron nitrosyl-hemoglobin in a mammal, comprising administering to the mammal a composition comprising hemoglobin and about 10 millimolar phosphate and about 90 millimolar borate.

12. A method for effecting NO delivery in a mammal, comprising administering to the mammal a composition comprising hemoglobin and a physiologically compatible buffer that promotes cooperative binding of NO to hemoglobin.

13. A method for treating ischemia in a mammal, comprising administering to the mammal a composition comprising hemoglobin and a physiologically compatible buffer that promotes cooperative binding of NO to hemoglobin.

14. A method for treating sickle cell disease in a human, comprising administering to the human a composition comprising hemoglobin and a physiologically compatible buffer that promotes cooperative binding of NO to hemoglobin.

15. A method for treating sickle cell disease in a human, comprising administering to the human a composition comprising hemoglobin, about 10 millimolar phosphate, and a composition comprising NO gas by inhalation.

16. A method for treating sickle cell disease in a human, comprising administering to the human inhaled oxygen and NO, and a composition comprising hemoglobin, wherein the inhaled oxygen is manipulated to achieve a desired concentration of SNO-hemoglobin in the blood.

17. A method for treating sickle cell disease in a human, comprising administering to the human a composition comprising hemoglobin, about 10 millimolar phosphate, and inorganic nitrite at a ratio of about 1 per 100 hemoglobin molecules.

18. A method for delivering NO to a mammal, said method comprising isolating biologically compatible erythrocytes, deoxygenating the erythrocytes, adding NO as dissolved gas to the erythrocytes, oxygenating the erythrocytes, and administering the erythrocytes to the mammal.

19. A method for inhibiting NO release from red blood cells in a mammal, said method comprising administering to the mammal an effective amount of an inhibitor of the transport function of AE1.

20. The method of Claim 19 wherein the inhibitor is selected from the group consisting of: phenylglyoxal, 1,3-cyclohexanedione, 1,4-cyclohexanedione, niflumic acid, 2,4-dinitrofluorobenzene, 2-[(7-nitrobenzofurazan-4-yl)amino]ethanesulfonate, 2,4,6-trichlorobenzenesulfonate, 1,2-cyclohexanedione, dipyridamole, 4,4'-diisothiocyanatostilbene-2,2'-disulfonic acid, *p*-nitrobenzenesulfonate, 4,4'-dinitrostilbene-2,2'-disulfonate, and *p*-aminobenzenesulfonate.

21. A method for scavenging NO and free radicals in a mammal, said method comprising administering to the mammal an effective amount of an inhibitor of AE1 anion transport function.

22. A method for treating an inflammatory condition in a mammal, said method comprising administering to the mammal an effective amount of an inhibitor of AE1 anion transport function.

23. A method for preserving red blood cells, said method comprising adding a solution comprising dissolved NO gas to a composition comprising red blood cells, to a final ratio of about 1:4000 to 1:50 NO:heme.

24. A method for decreasing the release of nitric oxide biological activity from red blood cells in a mammal, comprising administering to the mammal an effective amount of a composition comprising an inhibitor of carbonic anhydrase II activity.

25. The method of Claim 24, wherein the inhibitor of carbonic anhydrase II activity is selected from the group consisting of: (4*S-trans*)-4-(ethylamine)-5,6-dihydro-6-methyl-4*H*-thieno[2,3-6]thiopyran-2-sulfonamide 7,7-dioxide

monohydrochloride, 4,5-dichloro-1,3-benzendisulfonamide, acetazoamide, methozolamide, MK-927, L-662,583, and L-693,612.

26. A method for treating a medical disorder mediated by nitric oxide, said method comprising administering to a mammal a composition comprising SNO-hemoglobin and an agent that facilitates the release of nitric oxide from SNO-hemoglobin, wherein the agent is selected from the group consisting of:

a) SEQ ID NO:1;

b) SEQ ID NO:3;

c) SEQ ID NO:4;

d) a mimetic of any of a), b) or c); and

e) a peptide with one or more amino acid substitutions, deletions or additions compared to any of a), b) or c).

27. A method for restoring red blood cells in a mammal, comprising administering to the mammal a composition comprising red blood cells which have been treated with NO gas, the red blood cells thereby comprising NO at a concentration of greater than about 0.3 μM.

28. A method for determining the predominant physiological effect of a blood sample from a patient, comprising the steps of:

a) obtaining EPR or UVspectra of iron-nitrosyl hemoglobin derivatives formed by incubation of limiting NO with hemoglobin at various degrees of oxygen saturation;

b) determining from the results in a) whether the composition shows non-cooperativity or cooperativity in binding of NO to the hemoglobin; and

c) if the composition shows cooperativity, then assaying the composition at an oxygen saturation at which the hemoglobin is approximately 99%

oxyhemoglobin and 1% deoxyhemoglobin, under limiting NO concentration, to determine whether *S*-nitrosohemoglobin or iron nitrosyl-hemoglobin is greater;

wherein, the composition shows cooperativity, the most prevalent species of NO-modified hemoglobin is *S*-nitrosohemoglobin, and the predominant physiological effect of the composition is vasodilation; and further comprising the step of administering to the patient added thiol.

29. A method for treating sickle cell disease in a patient, said method comprising administering to the patient hemoglobin and inhaled nitric oxide and oxygen, wherein the amount of oxygen and NO administered is determined by measurement of SNO-hemoglobin.